

# Effects of repeated exposure to malathion on growth, food consumption, and locomotor performance of the western fence lizard (*Sceloporus occidentalis*)

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*Repeated exposure of western fence lizards to malathion caused reduced arboreal performance and some mortality but growth, food consumption, and terrestrial performance were not affected.*

## Abstract

Effects of repeated pollutant exposure on growth, locomotor performance, and behavior have rarely been evaluated in reptiles. We administered three doses of malathion (2.0, 20, or 100 mg/kg body weight) to western fence lizards (*Sceloporus occidentalis*) over an 81 day period. Eight and 23% mortality occurred at 20 and 100 mg/kg ( $p = 0.079$ ) and 85% of lizards in the 100 mg/kg group exhibited clinical symptoms of poisoning. Growth, food consumption, body condition index, and terrestrial locomotor performance were not significantly influenced by malathion. However, arboreal sprint velocity was significantly reduced in lizards receiving 100 mg/kg. Fifty percent of lizards in the 100 mg/kg group also refused to sprint in the arboreal setting ( $p = 0.085$ ). Based on these results, arboreal locomotor performance was the most sensitive metric of exposure we evaluated. Further study of compounds such as malathion is warranted due to highly variable application rates and exposure scenarios.

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**Keywords:** Repeated exposure; Lizard; Locomotor performance; Malathion; Reptiles

## 1. Introduction

Environmental pollutants are one of the suspected factors contributing to the global decline of herpetofauna (Gibbons et al., 2000). In amphibians, exposure to pollutants has been shown to influence fitness-related traits such as growth, locomotor performance, and behavior (Boone et al., 2001; Bridges, 1997, 1999; Fordham et al., 2001; Hopkins et al., 2000;

Lefcort et al., 1998; Raimondo et al., 1998). However, with the exception of a few recent papers (Bain et al., 2004; DuRant et al., 2007b; Holem et al., 2006; Hopkins et al., 2005a; Hopkins and Winne, 2006), the effects of pollutant exposure on fitness-related traits of reptiles is largely unknown (Campbell and Campbell, 2002; Hopkins, 2006) and reptiles remain the least studied vertebrates in ecotoxicology (Hopkins, 2000).

Repeated exposure to pesticides and other environmental contaminants could influence the locomotor performance, growth, and ultimately the fitness of lizards. Locomotor performance is important to lizards due to its influence on prey capture (Webb, 1986), predator evasion (Christian and Tracy, 1981; Huey and Dunham, 1987; Vanhooydonck and Van Damme, 2003) and social dominance (Garland et al., 1990).

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A wealth of information exists on locomotor performance of lizards (Aerts et al., 2000), yet to our knowledge only two studies (DuRant et al., 2007b; Holem et al., 2006) have evaluated the effects of pollutant exposure on lizard performance and no studies have thoroughly examined growth which influences many fitness-related parameters such as juvenile survival rate, age at sexual maturity, as well as territory establishment and defense in lizards (Clobert et al., 2000; Cox et al., 2003).

To evaluate whether environmental contaminants can impact fitness-related traits in lizards, we exposed western fence lizards (*Sceloporus occidentalis*) to malathion (S-[1,2-bis(ethoxycarbonyl)ethyl] O,O-dimethyl phosphorodithioate), a broad-spectrum organophosphate (OP) insecticide that acts via cholinesterase (ChE)-inhibition. Malathion is applied to control insects such as the boll weevil (*Anthonomus grandis grandis*), Mediterranean fruit fly (*Ceratitis capitata*), and mosquito (family Culicidae). In 2001 (the most recent year for which data were available), the United States Environmental Protection Agency estimated that 73 million pounds (active ingredient) of OP insecticides were applied in the US, accounting for 70% of the total insecticide use (Donaldson et al., 2004). Twenty to twenty-five million pounds (active ingredient) of malathion were applied that year, making it the most widely used OP in the US in 2001. Non-target invertebrates (e.g., ants, spiders) are often exposed to insecticides following broadcast application techniques (e.g., large-scale spray applications) (Wallace, 1992), placing terrestrial vertebrates such as lizards at risk due to ingestion of contaminated prey or direct contact with recently-sprayed substrate. Repeated exposure to malathion should result in a buildup of acetylcholine in neuromuscular junctions (Fukuto, 1990) and the resulting disruption of normal nervous system function could affect locomotor performance and other critical parameters such as food consumption, which could influence growth. The objectives of this experiment were to evaluate the effects of repeated malathion exposure on growth, food consumption, and terrestrial and arboreal locomotor performance in *S. occidentalis*.

## 2. Materials and methods

### 2.1. Lizard natural history and husbandry

*Sceloporus occidentalis* is a small (12–20 cm total length) diurnal lizard that occurs in many habitat types throughout the western United States. It is an opportunistic predator, frequently preying upon ants, beetles, termites and other invertebrates (Rose, 1976). *Sceloporus occidentalis* was selected as a model for toxicological study based on its role as an active consumer in the terrestrial food web, capacity to occupy diverse habitats, and life history traits that make it desirable for laboratory studies (e.g., rapid maturation rate, high survival rate; Talent et al., 2002).

Juvenile male western fence lizards were obtained from a breeding colony at Oklahoma State University and shipped to the Savannah River Ecology Laboratory (Aiken Co., SC, USA) in early 2005. Male lizards were used to avoid the potential confounding effects of gravidity on performance (Sinervo et al., 1991). The parental stock of lizards used to establish the breeding colony originated from the San Joaquin Valley, CA, USA (Talent et al., 2002). Lizard husbandry was identical to Hopkins et al. (2005b). Briefly, lizards were housed individually in plastic cages arranged in a rack system ( $n = 15$  cages/rack). Full spectrum bulbs (15–40 W) suspended over each cage provided

a ~29–40 °C thermal gradient with a 10:14 h light:dark photoperiod. Because the thermal gradient varied slightly with vertical cage position on the rack, cages were rotated every 5 days to equalize time spent on each level of the rack.

### 2.2. Malathion administration and dose levels

Pesticides such as malathion are routinely applied to crops several times per growing season (Ragnarsdottir, 2000). In our experiment, each lizard was administered a total of three doses of malathion or corn oil (vehicle control) over an 81 day period (single dose every 27 days). The dose interval selected was meant to approximate a monthly application regime. Fifty-two male lizards were randomly distributed among four treatment groups ( $n = 13$  per group). Each lizard was then assigned a “blind ID” to prevent bias during performance trials. Lizards in three treatment groups received single doses of malathion via oral gavage (for gavage technique see Holem et al., 2006) at 2.0, 20, or 100 mg/kg body weight (bw) every 27 days while the fourth treatment group received a single gavage of corn oil (vehicle control). Gavage volumes were calculated based on lizard mass collected immediately prior to dosing. A previous experiment with *S. occidentalis* demonstrated that true controls and gavage controls did not differ in sprint velocity (Holem et al., 2006). Thus, only vehicle (corn oil) controls were used in this experiment. To our knowledge, only one study (Stromborg et al., 1984) has reported malathion residue levels on invertebrates, which complicated the selection of doses for our experiment. In the Stromborg study, malathion applied at the rate of 0.61 kg active ingredient/ha yielded peak residue levels of 2.8 µg/g (wet weight) on grasshoppers sampled 30 h post-spray. Malathion application rates vary widely, however, and application rates as high as 1.36 kg Active Ingredient/ha have been reported in the literature (Tillman and Mulrooney, 2001). Based on these application rates and residue levels, we estimated that a 10 g lizard could consume up to 6.2 µg of malathion for every gram of prey consumed. To account for wide-ranging application rates, the potential for cumulative exposure, and the fact that lizards can easily consume more than 1 g of prey before the half-life of malathion (1–9 days in soils and on vegetation) is reached in the field, 2.0 mg/kg was selected as our low dose and 20 and 100 mg/kg as our intermediate and high doses, respectively. These latter concentrations have not been observed in field-collected prey, but are sublethal when administered only once, and are within the range recently examined in the literature (Holem et al., 2006). Gavage volumes were regulated using a 2–20 µl Eppendorf® reference pipette and varied according to lizard mass (volumes ranged from 8.9–16 µl). Lizards were immediately returned to their cages after dosing and were observed for clinical symptoms of organophosphate poisoning (e.g., obvious limb and body tremors) approximately every 30 min for 4 h and again prior to 24 h post-dose performance trials.

### 2.3. Food consumption and growth measurements

Initially, lizards were given a ration of crickets equal to 3% of their body mass on all days that food was offered; lizards were fasted the day before and during performance and dose sessions. This ration was increased to 5% after day 19 to improve growth in all treatment groups (see Fig. 1) and remained at 5% for the rest of the experiment. Crickets were counted and massed to the nearest 1.0 mg before each feeding and rations were adjusted according to lizard mass (g), which along with snout-vent length (SVL-mm), was measured approximately every 5th day (17 times total). Lizards were fed by 1100 h, and uneaten crickets were removed and massed at 1730 h. The vitamin supplements Herpivite (dusted onto crickets) and calcium powder (both from RepCal®) were provided throughout the study.

### 2.4. Terrestrial performance

Terrestrial performance was evaluated by measuring the velocity of lizards on a 2.3 m sprint track using methods identical to Holem et al. (2006). Lizards were brought to their optimum activity temperature (34 °C, Adolph, 1987; Brattstrom, 1965) and hand-chased down a photocell-lined track that was interfaced with a laptop computer and velocities were calculated over each 0.2 m interval. Each lizard was raced three times in succession and maximum

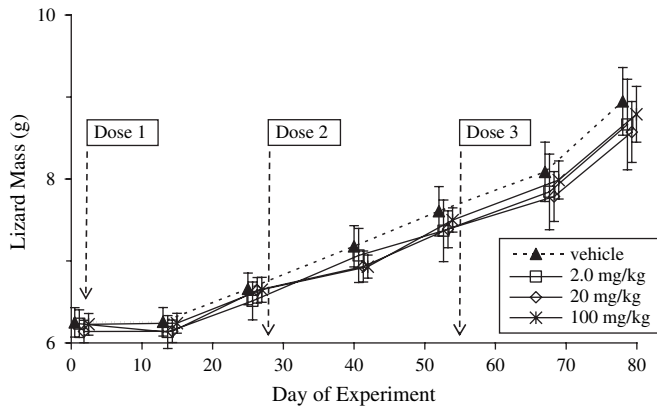


Fig. 1. Change in mass (g) of fence lizards. Error bars are  $\pm 1$  standard error of the mean. Treatment means are offset slightly on the x-axis for clarity.  $N = 13$  for vehicle control and 2.0 mg/kg groups;  $N = 10$  for 20 mg/kg group due to injury and mortality;  $N = 10$  for 100 mg/kg due to mortality.

velocity was estimated using two methods. In the first method, the fastest 0.2 m interval of the three sprints was considered the maximum terrestrial velocity (MTV), a method consistent with the ecological and evolutionary literature (Bennett, 1980; Holem et al., 2006; Huey and Dunham, 1987; Sinervo and Losos, 1991). However, our recent work demonstrated that use of the single fastest velocity failed to capture the overall performance of lizards exposed to contaminants, which may generally run more slowly during the three races and only attain their MTV once, presumably because of compromised endurance (Holem et al., 2006). Therefore, we also calculated the average of the fastest 0.2 m interval from each of the three sprints to produce the mean maximum terrestrial velocity (MMTV), a technique that better accounts for intra-individual variability in performance.

### 2.5. Arboreal performance

Arboreal performance was evaluated on a 1.6 m artificial branch (mesh-covered dowel rod, 2.5 cm diameter; Losos and Irschick, 1996) that was suspended  $\sim 2$  m above the ground. Arboreal velocity, number of jumps and falls, and refusal to traverse the 1.6 m dowel rod were determined. The rod was marked at 10 cm increments and a thin strip of orange tape was placed at the beginning of each quartile (0, 40, 80, and 120 cm). Lizards were released at the 0 cm mark and hand-chased the length of the rod towards a hidebox positioned at the end of the rod. Traverses were given one of four designations: “complete” (lizard advanced the length of the rod without jumping or falling off), “jump” (lizard voluntarily leapt from the rod before reaching the end), “fall” (lizard fell off before it reached the end of the rod), or “refusal” (lizard refused forward progress on the rod after 20 taps on the tail). Due to the difficulty in determining the exact position of a jump or fall, lizards were placed at the quartile mark that preceded the jump or fall (e.g., jumped at  $\sim 55$  cm, restarted at 40 cm mark) and were hand-chased down the remaining length of the rod (to obtain velocity data). The sequence and number of jumps/falls was counted for each traverse. Traverses were videotaped (Panasonic® model PV-DV73) and arboreal velocity was calculated by counting the number of video frames it took to traverse each 0.2 m interval and dividing by the frame rate (30 frames per second) (Hopkins et al., 2005a). Each lizard was allowed 2 traverses of the rod and maximum velocity was estimated similarly to terrestrial velocity (i.e., maximum arboreal velocity (MAV) and mean maximum arboreal velocity (MMAV)).

Terrestrial and arboreal performance trials were conducted a total of 10 times (per lizard) throughout the experiment. In each trial, terrestrial performance was assessed first then each lizard was returned to its cage for 4 h before assessing arboreal performance. Performance trials were held 24 h before the first malathion dose (for baseline data) and 24, 288, and 576 h (1, 12, and 24 days) after each malathion dose. Performance of *S. occidentalis* was not different 4 h after exposure to malathion and Pb in a previous experiment (Holem et al., 2006), thus 24 h was selected as the first post-dose performance trial in

the current experiment. This also made it possible to evaluate terrestrial and arboreal performance endpoints in the same day.

### 2.6. Statistical analysis

Prior to statistical analysis, data were tested for normality and homoscedasticity using Ryan Joiner and Bartlett’s tests, respectively. All velocity and body size data were nearly normally distributed and variance was similar among treatments, but other data required transformation to meet assumptions of models. Lizard survival was compared among treatment groups using Fisher’s Exact Test. Three lizards died after receiving 100 mg/kg malathion doses (decreasing sample size to  $N = 10$ ). In addition, one lizard died after receiving 20 mg/kg malathion doses and two additional lizards (20 mg/kg group) were injured attempting to escape (decreasing sample size to  $N = 10$ ). Because repeated measures analysis requires no missing values, these lizards were omitted from all statistical comparisons and figures. Lizard growth and food consumption were compared among treatment groups using repeated measures analysis of variance (ANOVA) on  $\log_{10}$  (growth) and arcsin square-root (food consumption) transformed data, respectively. Final body condition index (BCI;  $(\text{Mass}/\text{SVL}^3 \times 10^6)$ , Romero and Wikelski, 2001) was compared among treatment groups using ANOVA on  $\log_{10}$  transformed data. Maximum terrestrial (MTV and MMTV) and arboreal (MAV and MMAV) velocities were compared among treatment groups using repeated measures analysis of covariance (ANCOVA; SAS Proc-mixed) with time as the repeated variable and lizard SVL (at the time of the performance trial) as the covariate. The percentage of lizards that jumped, fell, refused to sprint and failed to complete a traverse were compared among treatment groups over time using repeated measures ANOVA on arcsin square-root transformed data. Because qualitative observations suggested that refusals were much more common in individuals that had exhibited symptoms of OP poisoning, we also determined the percentage of lizards that refused at least one traverse during the 81 day study. These frequencies were then compared among treatment groups using a Fisher’s Exact Test.

## 3. Results

Eighty-five percent (11/13) of the lizards in the 100 mg/kg group exhibited clinical symptoms of OP poisoning (e.g., body/limb tremors, twitching; Chambers, 1992) within 4 h of dose administration. Symptoms generally subsided within 24 h. Although lizard survival was similar among treatment groups ( $p = 0.079$ ), a dose-dependent trend was observed. One-hundred percent survival ( $N = 13/13$ ) occurred in vehicle control and 2.0 mg/kg groups, while 91% ( $N = 10/11$ ) and 77% ( $N = 10/13$ ) survival was recorded in the 20 and 100 mg/kg groups, respectively. In the 20 mg/kg group, the single mortality occurred shortly ( $< 4$  h) after the third dose and single mortalities were recorded after each of the three doses in the 100 mg/kg group.

Despite clinical symptoms of toxicity, growth, food consumption, and BCI were similar among treatment groups. Lizard body size increased over time (Time:  $F_{16,672} = 178.95$ ,  $p < 0.001$ ) but was not influenced by malathion (Fig. 1; Treatment:  $F_{3,42} = 0.13$ ,  $p = 0.939$ ; Treatment  $\times$  Time:  $F_{48,672} = 0.36$ ,  $p = 1.00$ ). Similarly, the cumulative percentage of food that was refused increased over the course of the study (Time:  $F_{6,252} = 73.88$ ,  $p < 0.001$ ) but was not influenced by repeated malathion exposure (Fig. 2; Treatment:  $F_{3,42} = 0.43$ ,  $p = 0.735$ ; Treatment  $\times$  Time:  $F_{18,252} = 0.63$ ,  $p = 0.871$ ). Final lizard BCI ( $p = 0.749$ ) was similar among treatment groups (Vehicle (mean  $\pm 1$  SE):  $39.9 \pm 1.15$ ; 2.0 mg/kg:  $39.4 \pm 1.20$ ; 20 mg/kg:  $38.6 \pm 1.40$ ; 100 mg/kg:  $40.6 \pm 1.30$ ).

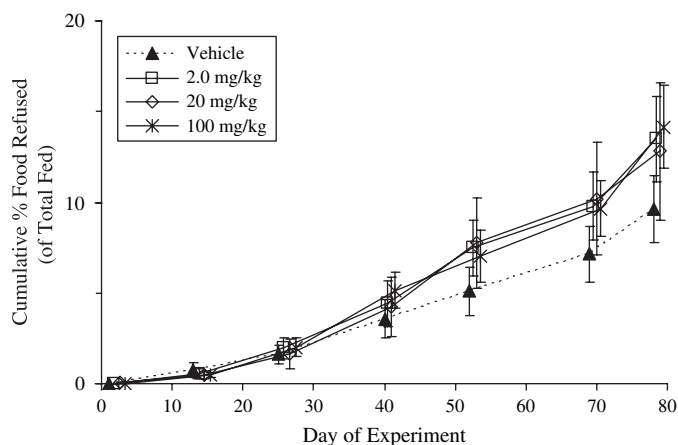


Fig. 2. Cumulative percentage of total food (crickets) refused by fence lizards. The mass of crickets refused was recorded to the nearest 1.0 mg on a daily basis. Error bars are  $\pm 1$  standard error of the mean. Treatment means are offset slightly on the x-axis for clarity.  $N = 13$  for vehicle control and 2.0 mg/kg groups;  $N = 10$  for 20 mg/kg group due to injury and mortality;  $N = 10$  for 100 mg/kg due to mortality.

### 3.1. Terrestrial performance

Although MTV fluctuated with time (Time:  $F_{9,378} = 4.13$ ,  $p < 0.001$ ), repeated exposure to malathion did not influence MTV (figure not shown; Treatment:  $F_{3,42} = 0.99$ ,  $p = 0.405$ ; Treatment  $\times$  Time:  $F_{27,378} = 0.88$ ,  $p = 0.637$ ). Likewise, MMTV was not influenced by malathion (Fig. 3A,  $p = 0.643$ ).

### 3.2. Arboreal performance

MAV and MMAV also fluctuated with time ((MAV) Time:  $F_{9,378} = 3.44$ ,  $p < 0.001$ ; (MMAV) Time:  $F_{19,798} = 13.53$ ,  $p < 0.001$ ), but only MMAV was influenced by repeated malathion exposure ((MAV), figure not shown; Treatment:  $F_{3,42} = 0.48$ ,  $p = 0.699$ ; Treatment  $\times$  Time:  $F_{27,378} = 1.17$ ,  $p = 0.260$ ; (MMAV) Fig. 3B, Treatment:  $F_{3,42} = 0.99$ ,  $p = 0.405$ ; Treatment  $\times$  Time:  $F_{57,798} = 1.70$ ,  $p = 0.001$ ). The percentage of incomplete traverses (both traverses combined) was not influenced by malathion ( $p \geq 0.177$ ; range: 8–35% control, 15–35% 2.0 mg/kg, 5–20% 20 mg/kg, 5–35% 100 mg/kg). Malathion did not influence the percentage of jumps and falls among treatment groups over time ( $p \geq 0.503$  in both cases). Marginal effects were observed among treatment groups in the percentage of arboreal refusals over time ( $p = 0.057$ , data not shown) and in the percentage of lizards refusing at least one arboreal traverse during the study (Fig. 4;  $p = 0.085$ ). Lizards in the 100 mg/kg treatment group were more than 6 times more likely than controls to refuse to run in the arboreal setting.

## 4. Discussion

The results of our study indicated that repeated exposure (three total doses, one every 27 days) to 100 mg/kg malathion can cause clinical symptoms of OP poisoning, induce

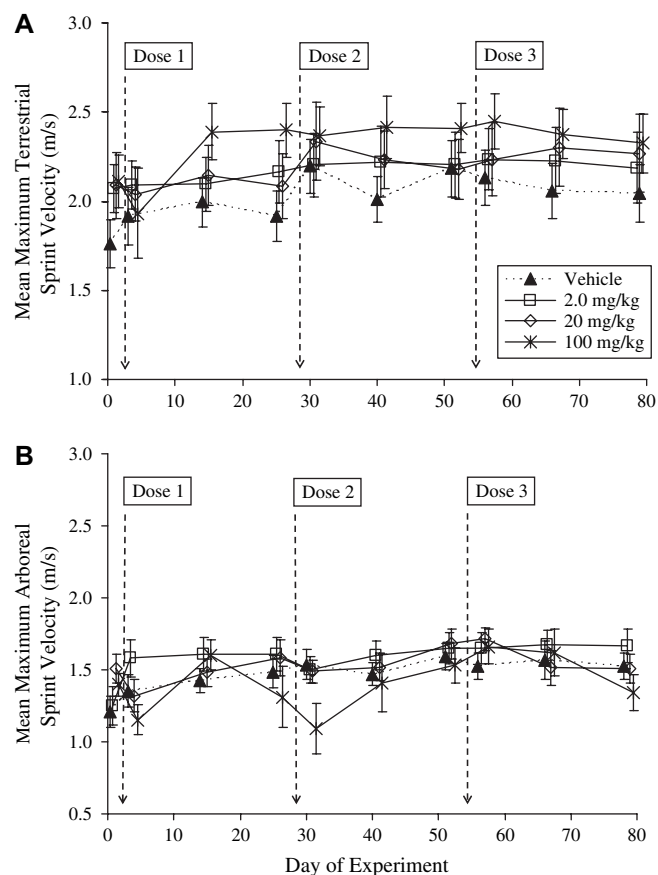


Fig. 3. Mean maximum terrestrial (A) and arboreal (B) sprint velocities (m/s) of fence lizards before and after oral administration of malathion on a milligram per kilogram, body weight basis. Three 2.3 m sprints (terrestrial) and two 1.6 m traverses (arboreal) were conducted per time period. Mean maximum terrestrial and arboreal velocity equaled the average of the fastest 0.2 m interval from each of the sprints and traverses. Error bars are  $\pm 1$  standard error of the mean. Treatment means are offset slightly on the x-axis for clarity.  $N = 13$  for vehicle control and 2.0 mg/kg groups;  $N = 10$  for 20 mg/kg group due to injury and mortality;  $N = 10$  for 100 mg/kg due to mortality.

mortality, and reduce arboreal locomotor performance of *S. occidentalis*. However, even at the highest concentration examined, malathion did not significantly influence growth, food consumption, or terrestrial locomotor performance in our study population. The rapid appearance (2–4 h post-dose) and disappearance ( $\sim 24$  h post-dose) of clinical symptoms of OP poisoning suggested that *S. occidentalis* recovered fairly rapidly from the doses of malathion administered in this study.

The effects of repeated pesticide exposure on growth and food consumption have been examined infrequently in lizards. In the current study, lizards exposed to malathion refused 3.2–4.5% more food than controls ( $p \geq 0.735$ ) and growth was not significantly influenced ( $p \geq 0.939$ ). Bain et al. (2004) reported no significant effect on the feeding rate of Australian central bearded dragons (*Pogona vitticeps*) exposed to a single dose of the OP fenitrothion. In contrast, Peveling and Demba (2003) reported decreased food consumption and body mass of fringe-toed lizards (*Acanthodactylus dumerili*) after exposure to the insecticide fipronil, which is moderately persistent and has been shown to accumulate in field-caught chameleons

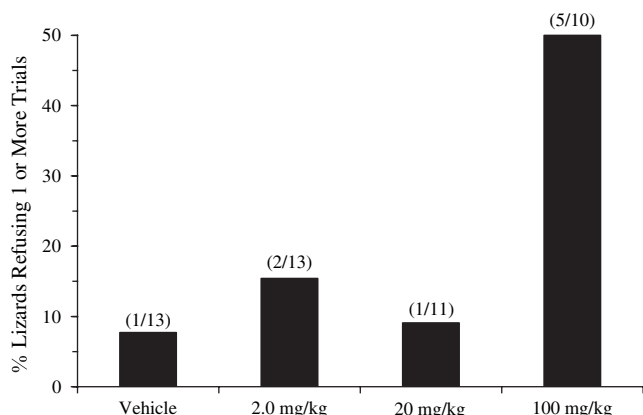


Fig. 4. Percentage of lizards in each treatment group that refused forward progress (after 20 tail taps) and were removed from the artificial branch on at least one arboreal traverse throughout the experiment. Two 1.6 m traverses were conducted during each performance session for a total of 20 traverses.  $N = 13$  for vehicle control and 2.0 mg/kg groups;  $N = 10$  for 20 mg/kg group due to injury and mortality;  $N = 10$  for 100 mg/kg due to mortality.

(Peveling and Demba, 2003). Most recently, *S. occidentalis* was found to exhibit reduced activity and food consumption following acute exposure to carbaryl, another ChE-inhibitor, but recovers within 96 h (DuRant et al., 2007a). The authors hypothesized that decreased energy expenditure from reduced activity could offset effects of reduced food consumption on lizard growth.

The results of our study and a recent study by DuRant et al. (2007b) suggest that effects of AChE-inhibiting pesticides on locomotor performance of *S. occidentalis* may be difficult to predict. In the current study, repeated malathion exposure did not significantly influence MTV or MMTV but a stimulatory response (9.5–24.5% increase in sprint speed compared to controls) was observed at the highest dose level (100 mg/kg) following the first dose of malathion. In previous studies, *S. occidentalis* exhibited a similar stimulatory response following single doses of malathion at 200 mg/kg (23% increase in terrestrial sprint velocity; Holem et al., 2006) and carbaryl at 2.5 and 25 mg/kg (17–33% increase in terrestrial sprint velocity; DuRant et al., 2007b). Conversely, single high doses of 250 mg/kg carbaryl resulted in a significant decrease in terrestrial sprint speed (DuRant et al., 2007b). In the current study, MAV was not influenced by repeated malathion exposure. However, MMAV was reduced in the highest dose group by up to 29% compared to controls 28 h after the second dose of malathion was administered (see Fig. 3B). This effect was detectable using MMAV because this metric captured the failure of lizards in the 100 mg/kg treatment to reach peak performance in both laps on the artificial branch. The DuRant et al. (2007b) study also noted decreases in arboreal velocities of *S. occidentalis* after exposure to 250 mg/kg carbaryl.

It is important to note that our experimental design may have prevented detection of effects of malathion on some fitness-related traits. Lizards were not fed on days they were dosed, or the day after (due to locomotor performance trials), thus food consumption was not quantified immediately following exposure to malathion. In a previous experiment, carbaryl-exposed

*S. occidentalis* consumed less food than control lizards in the 24 h following dose administration, but resumed normal feeding activity within 96 h of exposure (DuRant et al., 2007b). Due to logistical constraints, locomotor performance trials were started 24 h after each malathion dose; otherwise, lizards would have received no rest between dosing and terrestrial and arboreal performance trials. The most severe clinical symptoms of OP poisoning were observed 2–8 h after dose administration, suggesting the possibility that effects of malathion on food consumption and locomotor performance may have been missed or underestimated because these endpoints were not assessed during the time frame in which the most severe clinical symptoms were present.

Although the goal of this study was to examine fitness-related sublethal effects, mortalities in our highest dose group (100 mg/kg) suggested that *S. occidentalis* is within the range of sensitivity of other vertebrates (including lizards). In the current study, 9% (1/11) and 23% (3/13) mortality occurred in the 20 and 100 mg/kg treatment groups, respectively. These data support the findings of the only other study involving malathion and *S. occidentalis*, which reported 20% mortality after a single dose of 200 mg/kg (Holem et al., 2006). These data also reinforce the predicted malathion  $LD_{50}$  for subchronically exposed dwarf lizards (*Lacerta parva*) (169.8 mg/kg, Özelmas, 1993), but suggest that anoles (*Anolis carolinensis*) are more tolerant based on a predicted malathion  $LD_{50}$  of 2324 mg/kg (Hall and Clark, 1982). For comparison, reported mammal and avian malathion  $LD_{50}$ s were 1000 to 10,000 mg/kg (rat); 400 to <4000 mg/kg (mouse); and 167 and 1485 mg/kg for pheasants and mallards, respectively (Kamrin, 1997).

Behavior exhibited by *S. occidentalis* throughout this study suggests that behavioral responses may be useful indicators of neurotoxicant exposure. For example, in performance trials conducted 24 h (terrestrial) and 28 h (arboreal) post-dose, lizards exposed to 100 mg/kg malathion completed three 2.3 m sprints on the flat terrestrial sprint track with no apparent difficulty. In contrast, lizards often stopped forward progress on the arboreal beam, and 50% of lizards refused arboreal traverses at least once, a behavior never observed in terrestrial sprint trials (Fig. 4). This is consistent with the only other study that compared lizard terrestrial and arboreal performance following exposure to an AChE-inhibiting pesticide (DuRant et al., 2007b). Traversing the arboreal beam requires more coordination than traveling on the flat surface of the sprint track. Observed refusals suggest that lizards exposed to carbaryl and malathion were less likely to engage in these coordination-dependent activities than lizards in other treatment groups. Thus, endpoints that integrate both behavior and performance traits (e.g., time to flee from predators) may be more sensitive indicators of neurotoxicant exposure and should be considered in future studies.

Our study represents the first to examine the effects of repeated pesticide exposure on the locomotor performance, growth, and food consumption of lizards. Lizards exposed to the highest dose of malathion exhibited clinical symptoms of toxicity, a marginally significant reduction in survival, and impaired arboreal performance. Contrary to our predictions, food

consumption, growth, and terrestrial performance were not affected. The results of our study and previous work (DuRant et al., 2007b; Holem et al., 2006) suggest that performance measures, in particular arboreal performance, may be reliable indicators of exposure to AChE-inhibiting pesticides in *S. occidentalis*. However, many pesticides work via AChE-inhibition, and thus far, the majority of these compounds have not been evaluated for their effects on reptile health. Further research involving exposure scenarios likely encountered in the field (e.g., multiple doses) is recommended.

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